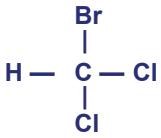
Toxicology and Carcinogenesis Studies of Bromodichloromethane (CAS NO. 75-27-4) in Male F344/N Rats and Female B6C3F1 Mice (Drinking Water Studies)



NIEHS

NTP
The National Toxicology Program
The Department of Health and Human Services

BDCM: Production/Human Exposure

- Not manufactured for commercial use
- Drinking water disinfection by-product: formed by the reaction of chlorine with naturally occurring organic matter in the presence of bromide
- Concentrations measured in drinking water: ~ 6 - 17 μg/L
- EPA's maximum contaminant level for total trihalomethanes in drinking water: 80 μg/L

BDCM: Study Rationale

- Although a previous gavage study showed that BDCM is carcinogenic at multiple sites in rats and mice, the US EPA nominated a drinking water exposure study
- To further characterize dose-response relationships between exposure to BDCM and neoplasia
- Studies limited to male rats and female mice because those sex/species groups showed higher incidences of large intestine neoplasms (MR) and hepatocellular neoplasms (FM) in the previous gavage study

BDCM: 3-Week Drinking Water Studies

Conc, mg/L	Survival	Body wt. gain, g	Av. daily water consumption, g		Av. daily dose, mg/kg
Male rats, N = 10			wk-1	wk-3	
0	10	65	16.8	18.2	-
43.7	10	63	15.7	17.5	6
87.5	10	63	15.2	17.7	12
175	10	59	13.5	15.0	20
350	10	57*	12.1	14.8	38
700	10	54**	10.3	13.2	71
Female mice, N = 10					
0	10	2.9	2.7	2.8	-
43.7	10	2.3	2.8	2.8	6
87.5	10	2.9	2.0	2.5	10
175	10	2.4	1.4	2.0	16
350	10	0.4**	1.1	1.7	32
700	10	1.2**	0.3	1.8	51

* P ≤ 0.05; **P ≤ 0.01

BDCM: 2-Year Drinking Water Studies

Conc, mg/L	Survival %	Av. Wt. at wk-52, g (% of control)	Av. daily dose mg/kg
Male rats			
0	58	492	-
175	57	479 (97)	6
350	58	479 (97)	12
700	52	472 (96)	25
Female mice			
0	72	61.2	-
175	74	58.1 (95)	9
350	66	58.1 (95)	18
700	78	55.6 (91)	36

2-Year Drinking Water Study of BDCM: Incidence (No./50) of Neoplasms in Female Mice

Lesion	0 mg/L	175 mg/L	350 mg/L	700 mg/L	Historical range
hepatocellular adenoma or carcinoma	30* (32)[a]	23 (27)	24 (27)	19* (24)	8 - 63 %
hemangio-sarcoma	8	2	0*	4	0 - 8 %

*p< 0.05

[a] expected rate based on body weight at wk-52

Conclusions

Under the conditions of this 2-year drinking water study there was *no evidence of carcinogenic activity* of bromodichloromethane in male F344/N rats exposed to target concentrations of 175, 350, or 700 mg/L. There was *no evidence of carcinogenic activity* of bromodichloromethane in female B6C3F$_1$ mice exposed to target concentrations of 175, 350, or 700 mg/L

Incidence of Kidney and Large Intestine Neoplasms in Male F344 Rats Exposed to BDCM

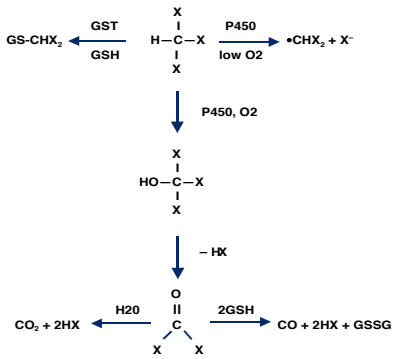
Gavage

	0	50 mg/kg	100 mg/kg
Kidney	0/50	1/50	13/50
Large intestine	0/50	13/50	45/50

Drinking Water

	0	175 mg/L	350 mg/L	700 mg/L
Kidney	0/50	0/50	0/50	0/50
Large intestine	0/50	0/50	1/50	0/50

Metabolism of THMs

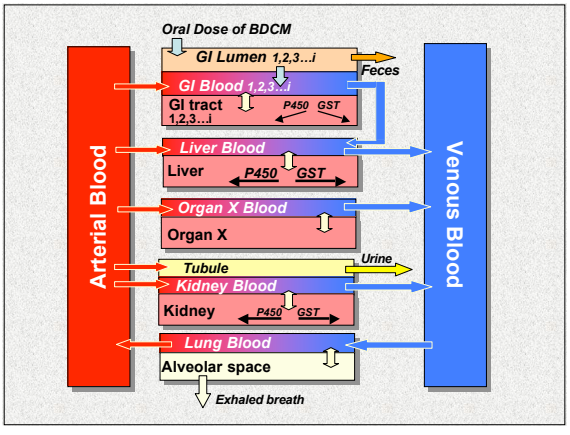


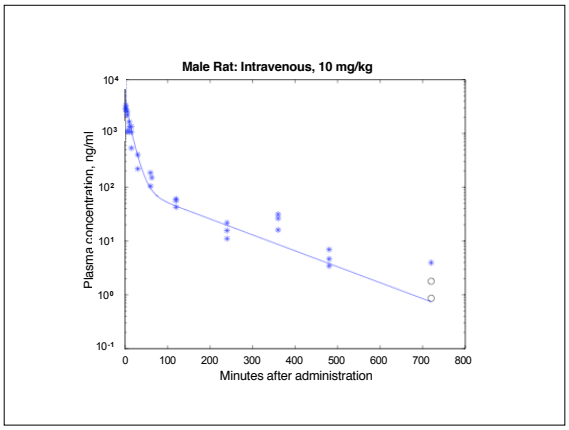
Characterization of BDCM in Male Rats and Estimation of Dose Metrics

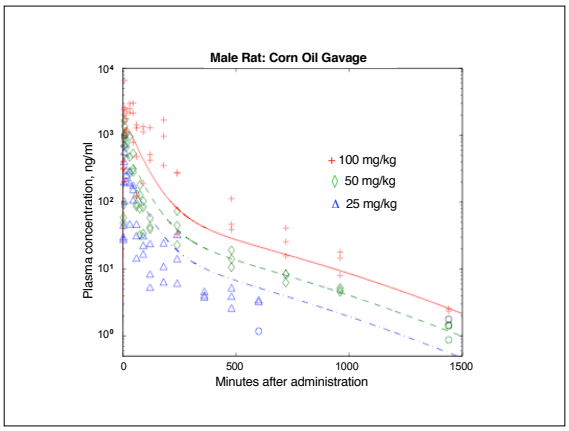
- Develop a PBPK model that characterizes the absorption, distribution, metabolism, and elimination of orally administered BDCM in male rats
- Fit this model to blood time-course data of BDCM
- Estimate potential dose metrics for neoplastic effects:
  - 24-hr blood AUC
  - Maximal and 24-hr cumulative metabolism via the GST pathway in kidney and large intestine
  - Maximal and 24-hr cumulative metabolism via the CYP450 oxidative pathway in kidney and large intestine

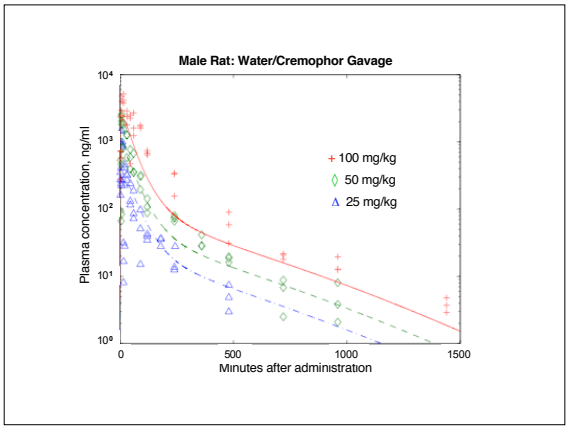
NTP Single-Dose TK Studies: Blood Time-Course Data of BDCM in Rats and Mice

Route	Doses, mg/kg
Intravenous	10
Gavage in corn oil	25, 50, 100
Gavage in water/cremophor	25, 50, 100




Male Rat: Intravenous, 10 mg/kg
Plasma concentration, ng/ml
Minutes after administration


Male Rat: Corn Oil Gavage
Plasma concentration, ng/ml
100 mg/kg
50 mg/kg
25 mg/kg
Minutes after administration


Male Rat: Water/Cremophor Gavage
Plasma concentration, ng/ml
100 mg/kg
50 mg/kg
25 mg/kg
Minutes after administration

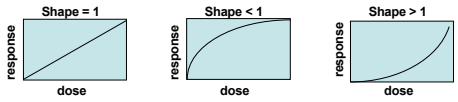
Behavior of BDCM in Male Rats after Oral Administration

- Absorption is rapid (plasma Tmax ~ 5-15 min) after gavage in corn oil or in aqueous vehicle
- Absorption occurs in stomach and intestines
- Metabolism occurs predominantly in the liver and mainly by the CYP450 pathway
- Partial saturation of the CYP450 pathway occurred with gavage administration but not with drinking water exposure
- Systemic availability of BDCM: 11-13% with gavage and 10-11% with drinking water exposure
- Greater relative flux through the GST pathway in the kidney and large intestine than in the liver

Strategy for Dose-Response Analyses

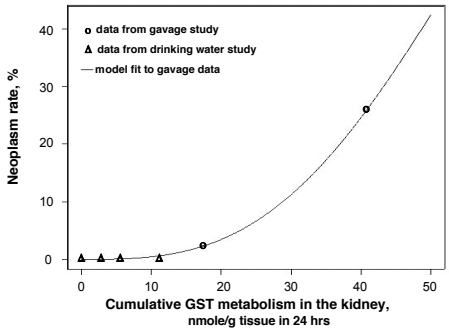
- Fit a Weibull dose-response model to the dose metrics from the PBPK model and the gavage cancer data

$$P(dose) = 1 - e^{-(intercept + scale \cdot dose^{shape})}$$

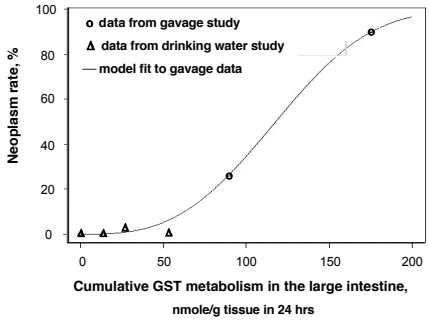


- Using the same model-based dose metrics for each target organ, predict neoplasm incidence in the kidney and large intestine with drinking water exposure
- Compare predicted neoplasm rates with observed rates
- Describe the shape of the dose-response curves for the combined gavage and drinking water tumor data

Tumor Response in the Kidney of Male F344 Rats versus Cumulative GST Metabolism



Tumor Response in the Large Intestine of Male F344 Rats versus Cumulative GST Metabolism



Observed and Predicted Incidence of Neoplasms in the Kidney and Large Intestine of Male F344 Rats Exposed to BDCM in Drinking Water

Exp conc, mg/L	Obs rate, %	Predicted neoplasm incidence, %				
		24 hr blood AUC	GST max	GST cumul	P450 max	P450 cumul
Kidney						
175	0	<0.1	<0.1	<0.1	<0.1	<0.1
350	0	<0.1	<0.1	<0.1	<0.1	<0.1
700	0	0.5	<0.1	0.6	<0.1	0.6
shape		3.2§	2.8§	2.6§	3.6§	1.9§
Large intestine						
175	0	0.3	<0.1	0.1	<0.1	<0.1
350	2	1.9	0.4	0.8	0.3	0.5
700	0	9.7*	3.5	6.3	3.5	6.0
shape		2.6§	3.2§	2.5§	4.3§	3.8§

*predicted incidence is different from observed, $P < 0.05$

§ shape parameter is different than 1, $P \leq 0.01$

Other Factors Influencing the Dose-Response

- Loss of BDCM (~ 20% in 3-4 days) from water bottles during exposure
- Blood AUC and metabolism via the GST pathway or the CYP450 pathway may not be the sole determinants of large intestine neoplasms
- Diet: 9.1% crude fiber in NTP-2000 diet used in the drinking water study, 3.4% crude fiber in NIH-07 diet used in the gavage study
- Route of exposure: dermal and inhalation exposures lack first-pass liver metabolism

Previous 2-year gavage studies of BDCM by the NTP provided clear evidence of carcinogenic activity in male and female F344/N rats and in male and female B6C3F$_1$ mice.

The different responses observed between the gavage and drinking water studies are attributed to differences in organ dosimetry by these routes of exposure and possible influences of dietary factors and differences in body weight on neoplasm development.